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Reducing dietary protein in pond production of hybrid striped bass (*Morone chrysops* × *M. saxatilis*): Effects on fish performance and water quality dynamics

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ABSTRACT

In previous work, we demonstrated that diets containing 40% digestible protein (DP) (45% crude protein) and 18% lipid supplemented with Met and Lys resulted in superior performance and nutrient retentions in hybrid striped bass (HSB) compared to less energy-dense diets when rearing HSB at temperatures similar to the summer growing period of the Southern U.S. The current work was aimed at vetting previous results in pond production of market-size HSB at commercial stocking densities to assess impacts on fish production indices and water quality parameters. Diets were formulated to contain digestible protein levels ranging from 41% to 35% DP and 15% crude lipid. The trial was conducted during the hottest portion of the growing season when impacts of feeding have the greatest influence on pond water quality. The 6-month pond (0.1 ha; 0.25 ac) study began with advanced juveniles (121.4 ± 3.21 g/fish; mean ± SD) in May 2013 and concluded with market sized fish in October 2013. Final fish size ranged from 448 ± 24 g to 1122 ± 46 g with food conversions ranging from 1.34–1.37. Results suggest that DP can be lowered from 41% DP to 35% DP by careful diet formulation while supplementing the first three limiting amino acids (Met, Lys Thr) without detrimental changes to body composition or nutrient retentions. Market size distributions, nutrient retention, and pond water quality dynamics in relation to test diets are also discussed.

1. Introduction

Evidence is accruing that population dynamics of natural moronid stocks are being impacted by global warming (Hanks and Secor, 2011). With temperatures predicted to rise approximately 0.2 °C per decade in mid-Atlantic to southern states (Najjar et al., 2010), there is mounting impetus to reexamine hybrid striped bass (HSB) nutrient requirements and feed formulas to maximize production efficiency, minimize excreted dietary nutrient impacts on water quality, and insure industry longevity in the face of climate change. Summer temperatures in sunshine hybrid striped bass (*Morone chrysops* × *M. saxatilis*) production ponds can reach extremes (29–33 °C). Because of peak feeding during this season, high total ammonia-nitrogen (TAN) and concomitantly lower dissolved oxygen typically reduce feed consumption in an episodic manner and increase the potential for stress, disease, and mortality.

Recommended production pond management practices call for total ammonia-nitrogen (TAN) concentrations not to exceed 2 mg/L (D'Abramo and Frinsko, 2008). Chronic or acute exposure to elevated un-ionized ammonia concentration can decrease fish growth or be toxic (Hargreaves and Kucuk, 2001). Control of TAN concentration in warm water aquaculture ponds is effected primarily by phytoplankton uptake (Hargreaves and Tucker, 1996; Hargreaves, 1998, 2006). Highest un-ionized ammonia concentration occurs when summertime afternoon pH in surface waters is high, and in catfish ponds is associated more with high pH than with TAN concentration (Tucker et al., 1984; Zhou and Boyd, 2015). Fish, however, always can move within the water column to areas where water quality conditions are more tolerable. The ability

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to manage nutrient loading in the pond through feed manipulation has been widely tested. One management tool currently utilized with pond reared fish is to not feed during periods when dissolved oxygen (DO) concentration is below or TAN concentration is above a threshold concentration. However, fish growth potential is reduced if feed input is restricted.

Nevertheless, there are potential advantages to reducing overall protein content of HSB feed, namely lower production costs, lower feed nutrient waste, and increased industry competitiveness, as long as fish performance and feed efficiencies are not degraded. Previous work has demonstrated that dietary protein can be reduced in fish diets by judicious supplementation of multiple amino acids. For example, Cheng et al. (2003) were able to reduce protein from 42 to 37% in fish meal based diets for rainbow trout when lysine, methionine, threonine and tryptophan were supplemented. Gaylord and Barrows (2009) found that the protein content of plant-based diets for rainbow trout can be reduced from 46 to 41.5% by supplementing lysine, methionine, and threonine on an ideal protein basis with no reduction in growth and an improvement in protein retention efficiency and muscle ratio. On the other hand, lysine supplementation alone (Gaylord et al., 2002) or with methionine (Li and Robinson, 1998) was insufficient to reduce protein levels in channel catfish diets without degradation of fish performance.

In a previous controlled tank study (Rawles et al., 2012) we investigated main and interactive effects of three digestible protein (DP) levels (33, 40, 47%), two lipid levels (10, 18%) and two ration levels (full fed: satiation, restricted: 80% of satiation) on growth, body composition, nutrient and amino acid retention, and ammonia and phosphorus excretion in hybrid striped bass (mean weight:75 g) reared at elevated temperature (30.5 ± 0.5 °C) to about 300–400 g average weight. Diets were balanced on an available amino acid basis to the profile of HSB muscle and supplemented with lysine and methionine at the equivalent of 330, 400, or 470 g/kg of muscle protein. We found that at elevated temperature, lower dietary fat (10%) resulted in lower weight gains and poorer feed conversions as well as higher ammonia excretion (per g N fed/kg BW) regardless of DP or ration level. Weight gain and final fish weight were highest at 47% DP/18% dietary lipid, but feed conversion, protein, energy and amino acid retention efficiencies were markedly poorer in the 47% DP diets regardless of lipid level. The 40/18 diet consistently outperformed the 33/18 diet in better growth and lower ammonia excretion as a function of N fed/BW, and nearly equaled the growth attained by fish fed 47/18 diet. Moreover, amino acid and protein retentions were nearly equal between the 33/18 and 40/18 diets and significantly better than the 47/18 diet. Hence, the results of that study suggested that a 40% DP/18% lipid diet formulated on an ideal protein basis would be the optimum among the six tested for summer HSB production up to 400 g final weight. The data also suggested that a producer desiring to reduce pond ammonia during high incidences of TAN that are typical during summer HSB pond production with the least compromise to production efficiency would be better served by feeding the 40/18 diet at a reduced level instead of switching to a lower protein diet.

Therefore, the current study aimed to extend the submarket-sized fish performance and water quality results of our previous tank study to market-sized HSB in pond production at commercial rearing densities, while simultaneously exploring the hypothesis that intact protein can be further lowered in production diets by supplementing the first three limiting amino acids (Lys, Met, Thr).

2. Materials and methods

2.1. Experimental design and diets

Three test diets were fed in a completely randomized design to quadruplicate ponds of hybrid striped bass for 167 days. The three commercial test diets(Table 1) were formulated at USDA/ARS – HKDSNARC (Stuttgart, AR USA) according to Gaylord and Rawles (2005) to contain one of three intact digestible protein (DP) levels (35, 38, 41%) and were supplemented with the first three limiting amino acids (Met, Lys, Thr) at an ideal protein level of 45% HSB muscle (Table 2). Apparent digestibility coefficients (ADCs) of gross nutrients and amino acid availability in the protein sources were taken from Barrows et al. (2011) and used to formulate the test diets. Protein in the diets was supplied by a combination of Select™ menhaden fish meal (MFM), soybean meal (SBM), petfood grade poultry by-product meal (PBM), poultry bloodmeal (BM), and poultry feather meal (FM), with a minor contribution from wheat flour. In order to limit response variability from ingredient effects, the following ratios of digestible protein from the various ingredients were held as constant as possible among formulas: animal: plant protein (2:1), MFM: SBM (1:1), MFM: PBM (1:1), BM: FM (1:1), and (MFM + SBM + PBM): (BM + FM) (6.98:1). In practice, however, actual ratios varied slightly; for example animal: plant protein varied from 2.01:1 in the 35% intact DP diet to 2.27:1 in the 41% intact DP diet. Because of differences between tabulated nutrient concentrations used for diet formulation (Barrows et al., 2011) and those in the specific ingredients used in this study, amino acid composition of the test diets varied somewhat from targeted levels (Table 2). For example, diet amino acid levels exceeded those in the ideal protein model for Ala (14–27% higher), Glx (4–8%), Gly (10–24%), Leu (8–19%), Lys (31–41%), Met (9–11%), Phe (16–20%), Thr (14–20%), and Val (8–19%); whereas, diet amino acid levels fell short of those in the ideal protein for Arg (30–38% lower; 4th limiting), Asx (8–17%), His (1–9%), Ile (5–14%), Ser (10–15%), and Tyr (8–12%). Similarly, test diets were formulated to contain 18% lipid supplied by a constant ratio (1:1.25) of fish lipid (8%) to poultry lipid (10%) from all ingredients. However, total lipid measured in the test diets fell short of the target by 3 percentage points (Table 1). Available phosphorus was targeted at 0.6% of diet to meet

Table 1
Formulation and composition (g/kg dry weight) of commercially extruded test diets fed to pond reared hybrid striped bass.

Ingredient[a]	Intact digestible protein (%)		
	35	38	41
Menhaden fish meal - Special Select™	146.2	162.0	177.8
Soybean meal	196.6	217.9	239.1
Poultry by-product meal – pet-food grade	163.0	180.6	198.2
Blood meal, spray dried poultry	33.6	37.3	40.9
Feather meal, hydrolyzed	29.3	32.5	35.6
Wheat flour	206.7	158.0	109.4
Menhaden fish oil	68.9	67.7	66.5
Poultry fat	72.3	69.4	66.4
Vitamin premix[b]	5.0	5.0	5.0
Mineral mix[c]	1.0	1.0	1.0
Monocalcium phosphate	13.3	8.9	4.4
Choline chloride 50%	6.0	6.0	6.0
Stay-C 35™	1.5	1.5	1.5
Potassium chloride	5.6	5.6	5.6
Sodium chloride	2.8	2.8	2.8
Magnesium oxide	0.5	0.5	0.5
DL-methionine	8.9	8.3	7.8
Lysine HCl	25.7	23.3	20.9
Threonine	13.0	11.8	10.6
Analyzed composition (dry weight basis)			
Crude protein (N × 6.25), g/kg	464.1	495.4	521.0
Crude fat, g/kg	153.8	151.4	153.1
Gross energy, MJ/kg	22.63	21.50	22.40
Moisture, g/kg	61.0	59.7	60.9
Ash, g/kg	100.8	105.6	106.9

[a] All ingredients were sourced by Skretting North America (North, Tooele, UT USA).
[b] Contributed, per kg of diet: vitamin A, 9650 IU; vitamin D, 6600 IU; vitamin E, 132 IU; vitamin K3, 1.1 mg; thiamin mononitrate, 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride, 13.7 mg; pantothenate, DL-calcium, 46.5 mg; cyanocobalamin, 0.03 mg; nicotinic acid, 21.8 mg; biotin, 0.34 mg; folic acid, 2.5 mg; inositol, 600 mg.
[c] Contributed, mg/kg of diet: zinc 40; manganese, 13; iodine, 5; copper, 9.

Table 2
Analyzed amino acid (A.A.) composition (g/kg dry weight) of hybrid striped bass muscle (ideal protein) and the test diets, percent difference (*in italics*) from ideal (IP), and the diet sum of squared amino acid deviations (SS Dev; ×10) from the IP concentrations.

Amino acid	Muscle[a] 450 g	Intact DP[b] 35	38	41
ALA	23.24	26.41 (*13.6*)	29.03 (*24.9*)	29.60 (*27.4*)
ARG	46.38	28.97 (*−37.6*)	31.58 (*−31.9*)	32.37 (*−30.2*)
ASX	49.82	41.32 (*−17.1*)	45.83 (*−8.0*)	46.11 (*−7.4*)
GLX	64.65	66.99 (*3.6*)	69.34 (*7.2*)	69.54 (*7.6*)
GLY	27.41	30.25 (*10.4*)	33.29 (*21.4*)	33.97 (*23.9*)
HIS	14.70	13.31 (*−9.4*)	14.36 (*−2.3*)	14.59 (*−0.7*)
ILE	21.09	18.21 (*−13.7*)	19.25 (*−8.7*)	20.02 (*−5.1*)
LEU	33.95	36.74 (*8.2*)	39.56 (*16.5*)	40.47 (*19.2*)
LYS	39.00	50.91 (*30.5*)	52.96 (*35.8*)	55.06 (*41.2*)
MET	14.89	16.29 (*9.4*)	16.48 (*10.7*)	16.08 (*8.0*)
PHE	18.95	22.04 (*16.3*)	22.33 (*17.9*)	22.79 (*20.3*)
SER	25.11	21.30 (*−15.2*)	21.80 (*−13.2*)	22.58 (*−10.1*)
THR	26.17	29.71 (*13.6*)	30.20 (*15.4*)	31.41 (*20.1*)
TYR	17.10	15.12 (*−11.6*)	15.63 (*−8.6*)	15.76 (*−7.9*)
VAL	23.66	25.45 (*7.6*)	27.22 (*15.1*)	28.22 (*19.3*)
SS dev (all A.A.)		60.45	61.09	69.16
SS dev (unsuppl. A.A.)[c]	44.82	39.72	40.48	

[a] Amino acid composition of 450 g hybrid striped bass muscle protein (dry-weight basis).
[b] Diet designations are intact % digestible protein (DP).
[c] SS Dev of the unsupplemented A.A. (without Lys, Met, and Thr).

the requirement of moronids (0.5–0.65%) according to NRC (2011, p. 164, Table 8-2).All ingredients were sourced by Skretting North America (North, Tooele, UT USA) and diets were manufactured by Skretting with commercial methods using a twin-screw cooking extruder to produce 3.5 mm floating pellets. Diets were bagged and shipped to HKDSNARC where they were stored in a temperature controlled (18–20 °C) feed room until use.

2.2. Fish, feeding, and pond management

The feeding trial was conducted in ponds at HKDSNARC over a 167-day period from May through October 2013, incorporating spring, summer, and fall temperatures of central Arkansas. Twelve 0.1-ha (0.25 ac) ponds were stocked with juvenile HSB originally obtained from Keo Fish Farm (Keo, AR USA) and reared on station. Fish were stocked May 11, 2013 with an initial weight of 121.4 ± 3.21 g/fish (mean ± SD; ≈0.27 lb./fish) at a rate of 2984 fish/acre (746 fish/pond). All fish were hand counted in order to ensure accurate stocking data. Initial fish biomass per pond were 90.4 ± 0.6 kg or 892.7 ± 5.5 kg/ha (796.8 ± 4.9 lb./ac). All ponds were fed a 48% crude protein/18% fat commercial hybrid striped bass diet (Cargill, Franklinton, LA USA) from stocking until June 6, 2013 (26 days) when the test diets arrived. Ponds were then randomly assigned to test diets (4 ponds/diet), which were subsequently fed from June 7 to October 26, 2013 (141 days). Both acclimation and test diets were fed by hand once daily to apparent satiation not to exceed 100 kg of feed/ha/d (i.e., 10 kg/pond; 88 lb./ac). Daily feed consumption was recorded as flat liters of feed consumed per pond, where a flat liter was defined as a liter cup filled with feed then leveled with a spatula. Flat liters were then converted to feed weight by adjusting for diet density as periodically determined in random samples ($N = 50$) of each diet measured throughout (every 60 days) the trial.

Ponds were filled with ground water from April 30 to May 6, 2013. Water was added as needed to replace losses to evaporation and seepage. Ponds were fertilized after filling and then as needed through July with chemical and organic fertilizer to promote and maintain a phytoplankton bloom. Urea fertilizer applications averaged 26 kg/ha, and total application averaged 91 kg/ha, not different ($P = 0.617$) among treatments. Average liquid fertilizer (9-37-0, N-P-K) application was 31 kg/ha and the same total amount (94 kg/ha) was added to each pond. Rice bran applications averaged 323 kg/ha, and the total applied (3292 kg/ha) did not differ among treatment ($P = 0.289$). Salt (2241 kg/ha) was added to all culture units to ensure chloride concentration exceeded 100 mg/L.

Each pond was equipped with an electric paddlewheel aerator (11.1 kW/ha) that was operated nightly from 2000 h to 0800 h. Dissolved oxygen and temperature in each pond were monitored continuously (10-sec scan rate) by a galvanic oxygen sensor (Type III, Oxyguard, Birkerød, Denmark) and a thermister (Model 109, Campbell Scientific, Logan, UT USA) connected to a datalogger (Model CR206, Campbell Scientific, Logan, UT USA).

Water samples were collected weekly from each pond at about 0800 h using a 90-cm column sampler. Sample pH was measured electrometrically. Water was filtered through 0.2-um pore size membrane filter and analyzed for nitrite-nitrogen (NO_2-N, diazotization), nitrate-nitrogen (NO_3-N, cadmium reduction), and soluble reactive phosphorus (SRP; PO_4-P, ascorbic acid method) using flow injection analysis according to manufacturer instructions (FIAlab 2500; FIAlab Instruments, Bellevue, WA USA). Flow injection analysis also was used to quantify total ammonia-nitrogen (TAN, NH_4-N) fluorometrically in filtered samples using the o-phthaldialdehyde method (Genfa and Dasgupta, 1989). Water samples were filtered through a 0.45-um pore size glass fiber filter for chlorophyll *a* analysis. Chlorophyll *a* (Chl *a*) was extracted in 2:1 chloroform:methanol from the phytoplankton retained on the filter, and the Chl a concentration in the extract was determined by spectroscopy (Lloyd and Tucker, 1988). Total alkalinity was measured on days 6, 76, and 132 by titrimetry (Eaton et al., 2005). The proportion of un-ionized ammonia for each event where TAN > 1.5 mg/L was calculated based on water temperature and pH (Emerson et al., 1975). Un-ionized ammonia-nitrogen concentrations were calculated using the measured early-morning pH and the minimum daily water temperature.

It is important to note that during the October 1 to 16, 2013, USA government shutdown, the one essential employee (M. McEntire) authorized to work ensured fish well-being, fed fish, and maintained pond water levels as needed. All other activities were suspended during that period. Animal care and experimental protocols used in this work were approved by the HKDSNARC Institutional Animal Care and Use Committee and conformed to USDA/ARS Policies and Procedures 130.4 and 635.1.

2.3. Fish and tissue sampling

Twenty randomly selected fish were collected and frozen at the beginning of the production trial for later analysis of whole body composition. During harvest (October 28 to 31, 2013), fish in each pond were harvested by seine, weighed in batches with a commercial fish loading basket and scale, and loaded into a commercial hauling tank and truck for transport to storage ponds. Fish from each pond were counted when off-loaded from the hauling tank onto a watered counting trough situated adjacent to the storage ponds. Weight distributions were estimated from samples taken during this enumeration process by selecting and weighing every 15th fish for a total of at least

50 out of 746 fish from each pond. Individual fish weights were used to assign fish from each pond into five industry-defined market classes (Wetzel et al., 2006) as follows: very small (VS; < 454 g; < 1 lb), small (S; 454–680 g; 1–1.5 lb), medium (M; 681–907 g; 1.5–2 lb), large (L; 908–1135 g; 2–2.5 lb), and jumbo/very large (VL; > 1135 g; > 2.5 lb). Subsets of approximately ten fish from each 50-fish sample were then randomly selected (every 4th fish) and frozen for the determination of body composition and nutrient and energy retentions. Similarly, approximately ten fish from each 50-fish sample were randomly selected (every 5th fish) for the determination of condition indices that included hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and muscle ratio (MR). Ponds were subsequently drained and fish that had evaded the seine were collected for inclusion in the final harvest data; these fish were not used for compositional data. Seine evasion averaged < 5 fish/pond, or < 1% of the final stock in each pond.

2.4. Diet and tissue chemical analyses

Whole bodies of fish from the start and end of the trial were rough-ground with a single pass through an industrial meat grinder (Hobart Inc., Troy, OH) fitted with a 24 mm grinder plate then stored at −20 °C until further processing. Rough-ground samples were subsequently passed two additional times through a 6 mm grinder plate and collected in a chilled stainless steel bowl to produce whole body homogenates. Whole body homogenates were pooled for each tank, thoroughly mixed, and three 100-g aliquots were packed into plastic trays and lyophilized (FreeZone® Triad™ freeze-drier, Model 7400030, Labconco, Inc., Kansas City, MO USA). Lyophilized samples were ground in a stainless steel laboratory blender to produce a uniform powder for analysis. Three aliquots of pooled, whole body sample from each pond were analyzed and averaged to obtain one representative value per replicate pond.

Proximate composition of diets and fish was determined according to standard methods (AOAC, 2006). Briefly, moisture was determined after drying in a convection oven (Isotemp 750F, Fisher Scientific, Hanover Park, IL USA). Protein (N × 6.25) was determined by the Dumas method using a LECO nitrogen analyzer (FP528, LECO Corporation, St. Joseph, MI USA). Total energy was determined by isoperibol bomb calorimetry (Parr1266, Parr Instrument Company Inc., Moline, IL USA). Lipid was determined by gravimetric quantification following acid hydrolysis (on feed samples only) and petroleum ether extraction (AOCS, 2009; Method AM 5-04) in an ANKOM XT15 lipid extractor (ANKOM Technology, Inc., Macedon, NY USA). Amino acids without tryptophan in diets and tissues were determined by the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MI, USA) using AOAC Official Method 982.30 E(a,b), chapter 45.3.05, 2006 and NIST Standard Reference Material 2389a (https://www-s.nist.gov/srmors/view_detail.cfm?srm=2389a). Glu and Gln were reported as Glx and Asp and Asn were reported as Asx since the acid hydrolysis procedure deaminates Asn and Gln to Asp and Glu, respectively. Protein, energy and amino acid retention efficiencies were estimated as: Protein, energy, or amino acid retention efficiency (RE) = (protein, energy, or amino acid gain × 100)/(protein, energy, or amino acid fed). In order to relate diet performance to amino acid profile of the diet relative to the ideal protein (IP) model, the distance between the amino acid profiles of the diets and 45% HSB muscle was defined as the sum of the squared deviations (SS Dev) from the IP concentrations. For example, from Table 2, the IP concentration for Ala is 23.24 g/kg in 450 g of HSB muscle, whereas the concentration of Ala measured in the 35 DP diet was 26.41 g/kg; the squared deviation from ideal for Ala in the 35 DP diet is then $(23.24–26.41)^2 = 10.05$. Similarly, the squared deviation from ideal for Arg in the 35 DP diet is $(46.38–28.97)^2 = 303.11$, etc., and finally for Val it is $(23.66–25.45)^2 = 3.20$. Hence, the sum of the squared deviations (SS Dev) from ideal for the 35 DP diet is $10.05 + 303.11 + \ldots + 3.20 = 604.5$ for all amino acids measured in the diet, and 448.2 for those amino acids measured but not supplemented (unsuppl.) in the diet (Table 2).

2.5. Statistical analysis

Fish production data, tissue composition, and nutrient retention efficiencies were analyzed by mixed model analysis of variance (ANOVA) using PROC MIXED in SAS version 9.3 (SAS Institute, Inc., Cary, NC USA). Body compositional indices data were analyzed by mixed model ANOVA with repeated measures (compound symmetry covariance structure). Differences among least squares means were evaluated using the DIFF option with the Bonferroni adjustment of *P* values in SAS in order to apply one of the most conservative means comparisons for reducing experiment-wise error rate. Percent data were log transformed prior to data analysis (Sokal and Rohlf, 1995). To determine if there were associations between diet and fish in each market size category, market size distributions were analyzed by PROC FREQ in SAS version 9.3 to produce Chi-square and likelihood ratios.

Water quality data were analyzed using PROC MIXED and differences among least squares means evaluated as described above. A subset of water quality data for the period June 11 to September 14, corresponding to "summer" was extracted for independent analysis using PROC MIXED and least squares means were evaluated for a linear trend using the CONTRAST statement. The PROC CORR procedure was used to calculate Spearman correlation coefficients within diet for weekly TAN and chlorophyll *a* concentrations and weekly averages of daily feed input. Differences among responses were declared significant at $P \leq 0.05$.

3. Results

3.1. Growth, feed performance, and condition indices

Total fish biomass per pond (520–540 kg), yield (5139–5342 kg/ha), survival (95.1–96.7%), FCR (1.34–1.37), weight gain (480–498%), average fish weight at harvest (732–734 g), and average maximum fish weight at harvest (1030–1122 g) were uncorrelated to diet DP level (Table 3). The average minimum fish weight at harvest was numerically larger (534 g) in ponds fed the 38% DP diet than in ponds fed the 35% (477 g) or 41% (448 g) DP diets (Table 3) though not statistically different ($P = 0.071$). Percent of fish at harvest that were over 680 g (1.5 lb) did not differ statistically ($P = 0.352$; 0.959 – linear; Table 3) but was 59% in ponds fed the 35% or 41% DP diets, as opposed to 71% in ponds fed the 38% DP diet. Maximum daily ration did not differ significantly ($P = 0.337$) among treatments and averaged 99, 95, and 90 kg/ha for the 35%, 38%, and 41% DP diets, respectively. At no time was daily feed ration restricted because of water quality. Total feed fed showed a slight linear increase ($P = 0.079$) with diet DP level (Table 3). Fish size at harvest was slightly ($P = 0.086$) more variable (higher CV) in ponds fed the 35% DP diet (Table 3). The distribution of market size fish fed the 41% DP diet was slightly more flattened ($P = 0.053$) about the mean (negative kurtosis) compared to distributions of market size fish fed the 35–38% DP diets; otherwise, size distributions did not appear significantly skewed ($P = 0.261$; 0.489 - linear) toward larger or smaller size classes with respect to diet DP (Table 3). Liver size (HSI) decreased linearly ($P < 0.001$), from 3.17% to 2.60%, with increasing diet DP (Table 3). Body fat content (IPF ratio; 8.6%–8.2%) and muscle ratios (50–51%) were not significantly influenced by diet DP level (Table 3).

3.2. Body composition and nutrient retention

Final whole body protein (16%), lipid (17%), energy (8.3–8.5 J/kg), moisture (62–63%) and amino acid content did not differ significantly among dietary treatments on a fresh weight basis (Table 4). Retention efficiencies of protein (PRE), Asx, Glx, Leu, Lys, Ser, and Val decreased

Table 3
Growth, feed performance, size distribution parameters, and composition indices of hybrid striped bass (initial weight: 121.4 ± 0.8 g/fish; mean ± SE) reared in ponds to market size on extruded diets containing one of three intact digestible protein (DP) levels (35, 38, 41%) and supplemented with Met, Lys, and Thr at an ideal protein level of 45% hybrid striped bass muscle[a].

Response[b]	Intact DP (%)			*ANOVA*	*Linear*
	35	38	41	*Pr > F*[c]	*Contrast*[d]
Total fish weight	520.0 ± 14.3	540.0 ± 16.5	533.3 ± 14.3	0.528	0.948
Yield	5139 ± 104 (4587 ± 140)	5342 ± 189 (4768 ± 169)	5271 ± 191 (4705 ± 170)	0.528	0.431
Survival	95.1 ± 1.2	96.7 ± 1.4	96.7 ± 1.2	0.302	0.170
Total feed	585 ± 11	616 ± 18	616 ± 9.0	0.536	0.079
FCR	1.34 ± 0.01	1.36 ± 0.03	1.37 ± 0.01	0.849	0.231
Gain	480.2 ± 7.4	490.0 ± 24.2	498.5 ± 14.3	0.681	0.370
Avg wt	736 ± 19	764 ± 22	732 ± 19	0.588	0.892
Max wt	1122 ± 46	1112 ± 54	1030 ± 46	0.338	0.152
Min wt	477 ± 24	534 ± 28	448 ± 24	0.071	0.964
% > 680 g	59 ± 5.8	71 ± 6.7	59 ± 5.8	0.352	0.959
CV	0.205 ± 0.008	0.176 ± 0.009	0.200 ± 0.008	0.086	0.889
Skewness	0.52 ± 0.15	0.62 ± 0.17	0.25 ± 0.15	0.261	0.489
Kurtosis	−0.03 ± 0.26a	0.31 ± 0.30a	−0.89 ± 0.26b	0.053	0.502
HSI	3.17 ± 0.15	2.81 ± 0.17	2.60 ± 0.15	0.080	< 0.001
IPF ratio	8.60 ± 0.18	8.64 ± 0.21	8.21 ± 0.18	0.213	0.121
MR	50.7 ± 0.57	50.5 ± 0.66	50.1 ± 0.57	0.782	0.221

[a] Values are least squares (LS) means of $N = 4$ replicate ponds of fish for diets 35% and 41% intact DP and 3 replicate ponds for 38% intact DP; least squares means in the same row with different letters are different ($P < 0.10$).
[b] Total pond weight (kg/pond) after 167 days; Yield, kg/ha (lbs/acre); Survival (%); Total feed (Kg, dry weight basis) consumed; FCR: feed conversion ratio = g dry feed consumed / g weight gained; Gain (%) = (final weight – initial weight) ∗ 100 / initial weight; Avg wt: average fish weight (g) at harvest; Max wt: maximum fish weight (g) at harvest; Min wt: minimum fish weight (g) at harvest; % > 680 g: percent of fish weighing > 680 g (1.5 lbs) at harvest; CV: coefficient of variation in fish size distributions; Skewness - denotes whether the fish size distribution is weighted toward smaller fish with fewer larger fish, i.e., right-tailed (+), heavier toward larger fish with fewer smaller fish, i.e., left-tailed (−), or symmetric (0) about the mean; Kurtosis - denotes whether the fish size distribution is flattened (−) about the mean, indicating fish are evenly distributed among size categories, or peaked (+) about the mean, indicating fish are bunched among few size categories around the mean; HSI: hepatosomatic index (%) = liver mass × 100 / fish mass; IPF: intraperitoneal fat (%) = intraperitoneal fat mass ∗ 100 / fish mass; MR: muscle ratio (%) = skinless fillet with rib mass ∗ 100 / fish mass.
[c] ANOVA, *Pr* > F. LS means in the same row with different letters are different ($P \leq 0.05$).
[d] Linear contrast, *Pr* > F. Linear effect of DP deemed significant at $P \leq 0.05$.

linearly with increasing diet DP. Retention efficiencies of Arg, His, and Ile also declined numerically with increasing DP but the trend was not strongly linear (*P* values 0.07–0.08). Nevertheless, the range in retention efficiencies among dietary treatments was less than two percentage points in all nutrients (Table 5). Retention efficiencies of energy (ERE), Ala, Gly, Met, Phe, Thr, and Tyr did not differ significantly with respect to diet DP level (Table 5).

Table 4
Whole body composition (fresh-weight basis) in hybrid striped bass (initial weight: 121.4 ± 0.8 g/fish; mean ± SE) reared in ponds to market size on extruded diets containing one of three intact digestible protein (DP) levels (35, 38, 41%) and supplemented with Met, Lys, and Thr at an ideal protein level of 45% hybrid striped bass muscle[a].

Response[b]	Intact DP (%)			*ANOVA*	*Linear*
	35	38	41	*Pr > F*[c]	*Contrast*[d]
CP	162 ± 3	163 ± 3	165 ± 5	0.879	0.609
Lipid	170 ± 4	170 ± 6	167 ± 4	0.871	0.625
Energy	8.30 ± 0.11	8.49 ± 0.02	8.45 ± 0.10	0.660	0.705
Moisture	625 ± 5	617 ± 1	616 ± 5	0.670	0.704
Ash	32.2 ± 1.2	32.9 ± 1.7	34.7 ± 2.7	0.717	0.413
ALA	11.44 ± 0.53	11.76 ± 0.13	11.87 ± 0.23	0.661	0.367
ARG	10.62 ± 0.35	10.91 ± 0.11	10.94 ± 0.21	0.626	0.359
ASX	14.86 ± 0.30	15.11 ± 0.06	14.98 ± 0.28	0.809	0.732
GLX	20.08 ± 0.19	20.39 ± 0.36	19.91 ± 0.24	0.463	0.758
GLY	15.50 ± 1.72	16.20 ± 0.51	16.71 ± 0.61	0.686	0.379
HIS	3.58 ± 0.10	3.57 ± 0.06	3.64 ± 0.07	0.824	0.567
ILE	6.42 ± 0.27	6.40 ± 0.11	6.39 ± 0.17	0.997	0.936
LEU	11.04 ± 0.40	11.08 ± 0.16	11.01 ± 0.23	0.985	0.968
LYS	12.97 ± 0.44	13.10 ± 0.14	12.94 ± 0.25	0.931	0.971
MET	4.39 ± 0.13	4.32 ± 0.08	4.39 ± 0.19	0.948	0.978
PHE	6.37 ± 0.21	6.37 ± 0.19	6.52 ± 0.11	0.783	0.514
SER	6.59 ± 0.06	6.73 ± 0.15	6.61 ± 0.04	0.555	0.921
THR	6.61 ± 0.14	6.74 ± 0.03	6.68 ± 0.13	0.782	0.680
TYR	5.00 ± 0.24	4.78 ± 0.29	5.03 ± 0.18	0.745	0.901
VAL	7.66 ± 0.27	7.91 ± 0.12	7.66 ± 0.17	0.666	0.973

[a] Values are least squares (LS) means of $N = 4$ replicate ponds of fish for diets 35% and 41% intact DP and 3 replicate ponds for 38% intact DP.
[b] Whole body composition includes CP: crude protein (g/Kg), lipid (g/Kg), energy (MJ/kg), moisture (g/Kg), and ash (g/Kg) and amino acids (g/Kg).
[c] ANOVA, *Pr* > F. LS means in the same row with different letters are different ($P \leq 0.05$).
[d] Linear contrast, *Pr* > F. Linear effect of DP deemed significant at $P \leq 0.05$.

Table 5
Whole body retention efficiencies (RE, %) of protein, energy, and amino acids in hybrid striped bass (initial weight: 121.4 ± 0.8 g/fish; mean ± SE) reared in ponds to market size on extruded diets containing one of three intact digestible protein (DP) levels (35, 38, 41%) and supplemented with Met, Lys, and Thr at an ideal protein level of 45% hybrid striped bass muscle[a].

Response[b]	Intact DP (%)			*ANOVA*	*Linear*
	35	38	41	*Pr > F*[c]	*Contrast*[d]
PRE	25.0 ± 0.7	23.5 ± 0.4	22.3 ± 0.9	0.104	0.029
ERE	37.4 ± 1.0	39.6 ± 1.1	36.7 ± 1.0	0.190	0.781
ALA	31.4 ± 1.9	29.5 ± 0.4	29.1 ± 0.5	0.443	0.209
ARG	26.6 ± 1.2	25.1 ± 0.5	24.5 ± 0.5	0.230	0.082
ASX	26.2 ± 0.6a	24.1 ± 0.7ab	23.5 ± 0.6b	0.030	0.010
GLX	21.8 ± 0.1	21.3 ± 0.8	20.5 ± 0.5	0.152	0.048
GLY	37.2 ± 5.2	35.6 ± 0.7	35.9 ± 1.3	0.995	0.961
HIS	20.0 ± 0.6	18.5 ± 0.8	18.4 ± 0.5	0.165	0.082
ILE	25.4 ± 1.2	23.8 ± 1.0	22.8 ± 0.8	0.214	0.071
LEU	21.7 ± 0.9	20.3 ± 0.8	19.5 ± 0.6	0.140	0.044
LYS	19.6 ± 0.7	19.0 ± 0.7	17.8 ± 0.5	0.152	0.048
MET	20.7 ± 0.7	19.9 ± 0.8	20.5 ± 1.1	0.826	0.825
PHE	21.2 ± 0.8	20.7 ± 0.9	20.7 ± 0.5	0.880	0.645
SER	22.2 ± 0.4a	22.0 ± 0.ab	20.7 ± 0.3b	0.079	0.035
THR	16.9 ± 0.4	16.8 ± 0.5	15.9 ± 0.4	0.218	0.110
TYR	24.3 ± 1.3	22.2 ± 1.7	23.2 ± 1.0	0.567	0.558
VAL	21.9 ± 1.0	21.2 ± 0.2	19.6 ± 0.6	0.115	0.039

[a] Values are least squares (LS) means of $N = 4$ replicate ponds of fish for diets 35% and 41% intact DP and 3 replicate ponds for 38% intact DP; least squares means in the same row with different letters are different ($P < 0.05$).
[b] Retention efficiencies include PRE: protein retention efficiency = g protein gain × 100/g protein fed, ERE: energy retention efficiency = kcal energy gain × 100/kcal energy fed, and amino acid retention efficiency = individual amino acid gain (g) × 100/individual amino acid fed (g).
[c] ANOVA, *Pr* > F. LS means in the same row with different letters are different ($P \le 0.05$).
[d] Linear contrast, *Pr* > F. Linear effect of DP deemed significant at $P \le 0.05$.

3.3. Fish size distributions

The Likelihood ratio ($P = 0.050$) statistic from frequency analysis of fish market size distributions showed a strong association between diet DP level and frequency of fish in each market size (Fig. 1). Specifically, there were more than expected medium (M) and very large (VL) fish and less than expected small (S) fish in ponds fed the 38% DP diet and more than expected VS fish in ponds fed the 35% DP diet. Additionally, there were fewer than expected VL fish in ponds fed the 41% DP diet (Fig. 1).

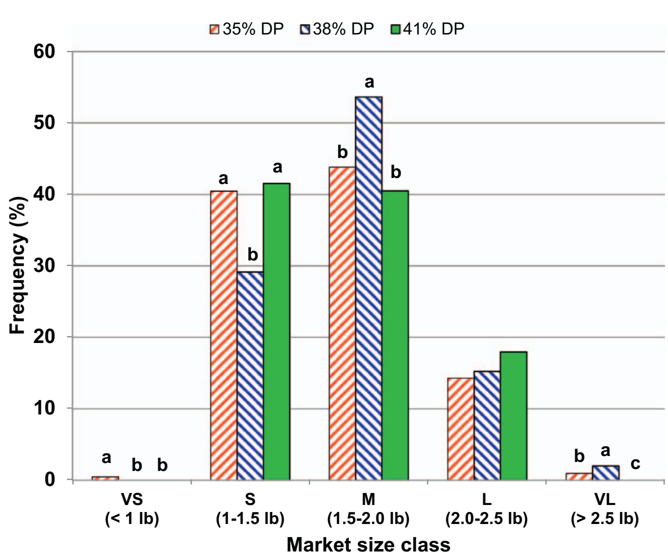

Figure 1. Market size class distribution by diet intact digestible protein (DP) level. Size classes are as follows: very small (VS; < 454 g; < 1 lb), small (S; 454–680 g; 1–1.5 lb), medium (M; 681–907 g; 1.5–2 lb), large (L; 908–1135 g; 2–2.5 lb), and jumbo/very large (VL; > 1135 g; > 2.5 lb). Frequencies within a size class with different letters are different according to the Likelihood ratio ($P = 0.050$) statistic.

3.4. Pond water quality dynamics

Mean, maximum, and minimum daily pond water temperatures throughout the study are shown in Fig. 2. Water temperature averaged 29.3 °C during the "summer" (June 11 to September 14) and the median "summer" temperature was 29.6 °C. Maximum daily water temperature ≥ 30.5 °C was recorded on 0 d in May, 17 d in June, 22 d in July, 25 d in August, 12 d in September, and 0 d in October.

Mean, maximum, and minimum DO concentrations, as percent saturation, throughout the study are shown in Fig. 3. Minimum DO concentration was ≥ 40% saturation except on 6 d in May and 12 d in June. Daily mean pond DO concentration was 7.4, 5.7, 6.2, 7.6, and 7.6 mg/L in June, July, August, September, and October, respectively.

Weekly water quality variable means did not differ significantly among treatments (Table 6). Mean weekly treatment TAN concentrations in all treatments fluctuated throughout the experiment (Fig. 4). Chlorophyll *a* mean weekly concentrations increased briefly in all treatments after stocking, then declined to < 100 mg/m^3 for about 5 weeks, after which mean concentrations increased (Fig. 5).

Weekly TAN exceeded 1.5 mg/L on average 3.25, 3.00, and 3.75 occasions for the 35 DP, 38 DP, and 41 DP treatments, respectively, but did not differ significantly ($P = 0.742$) among treatments. Mean (range) calculated un-ionized ammonia-nitrogen concentration, respectively, during these events was 0.23 (0.11–0.34), 0.23 (0.16–0.44), and 0.29 (0.19–0.47) mg/L NH_3-N. However, during "summer" (June 11 to September 14) the number of times weekly TAN exceeded 1.5 mg/L exhibited a positive linear trend ($P = 0.086$) with increased digestible protein (Table 6). No treatment differences were detected for the number of times weekly TAN exceeded 2.0 mg/L during the experiment ($P = 0.178$) or during "summer" ($P = 0.203$). Weekly TAN exceeded both threshold concentrations most frequently during June and the frequency of occurrence decreased during each subsequent month. The change in the quantity of feed fed on the day of the TAN spike, the previous day or the following 1 to 3 d was not correlated significantly with the TAN spike concentration.

Chlorophyll *a* concentrations exceeded 100 mg/m^3 during 15.4%, 20.0%, and 26.7% of the TAN > 1.5 mg/L events for the 35 DP, 38 DP, and 41 DP diets, respectively. Otherwise, chlorophyll *a* concentration was low and averaged 35.3, 47.5, and 42.4 mg/m^3, respectively.

Total ammonia-nitrogen and Chl *a* concentrations were correlated negatively for all diets ($r = -0.185$ and $P = 0.092$, $r = -0.320$ and $P = 0.011$, $r = -0.263$ and $P = 0.016$ for the 35 DP, 38 DP, and 41 DP diets, respectively). Total ammonia-nitrogen and soluble reactive phosphorus (SRP) concentrations were positively correlated ($r = 0.397$ and $P < 0.001$, $r = 0.383$ and $P = 0.002$, $r = 0.511$ and $P < 0.001$ for the 35 DP, 38 DP, and 41 DP diets, respectively). Soluble reactive phosphorus and Chl *a* were correlated positively for the 35 DP ($r = 0.276$, $P = 0.011$) and 41 DP ($r = 0.222$, $P = 0.042$) diets, but not the 38 DP diet ($r = 0.200$, $P = 0.120$). Feed input and SRP concentrations were correlated positively for all diets ($r = 0.485$ and $P < 0.001$, $r = 0.564$ and $P < 0.001$, $r = 0.475$ and $P < 0.001$ for the 35 DP, 38 DP, and 41 DP diets, respectively). Feed input and Chl a concentrations were correlated positively for all diets ($r = 0.292$ and $P = 0.007$, $r = 0.355$ and $P = 0.004$, $r = 0.426$ and $P < 0.001$ for the 35 DP, 38 DP, and 41 DP diets, respectively). No other correlations were detected.

4. Discussion

Fish yields (5139–5342 kg/ha), average weights at harvest (732–764 g), FCR (1.34–1.37), protein retention (PRE; 22–25%), and survival (95%–97%) were markedly better in this study than in several published hybrid striped bass (HSB) pond studies, but similar to

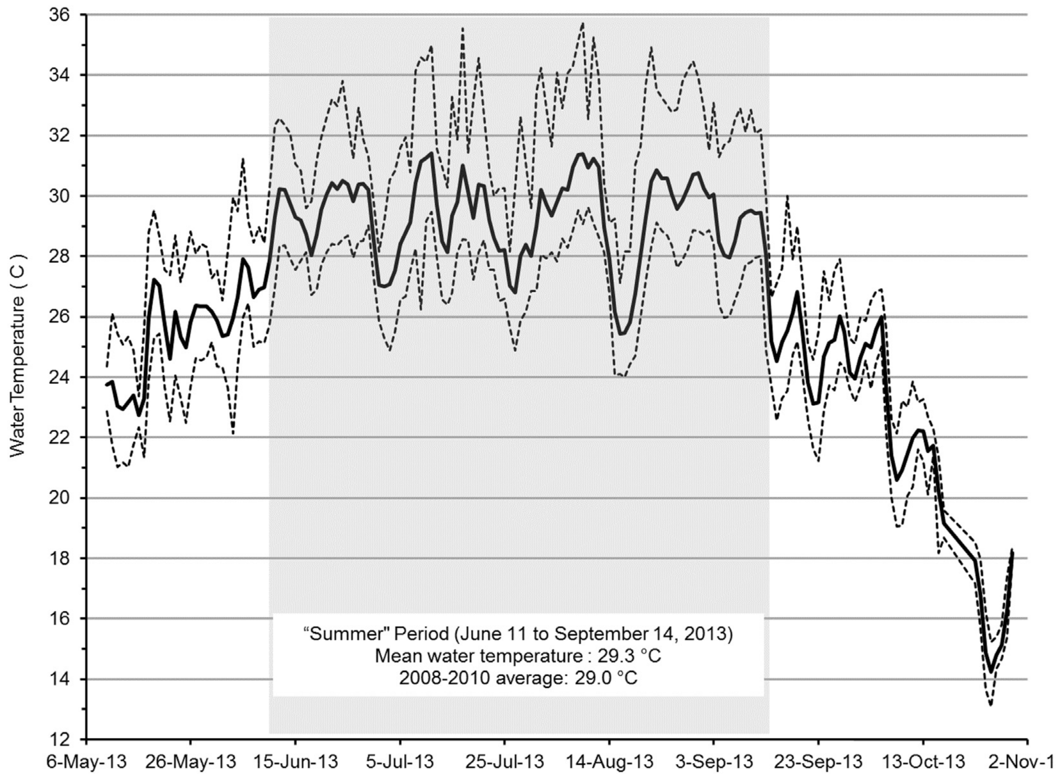

Figure 2. Mean (solid line) and maximum/minimum (dashed lines above/below mean) daily water temperatures in 0.1-ha earthen ponds stocked with hybrid striped bass fed diets with 35, 38, or 41% intact digestible protein.

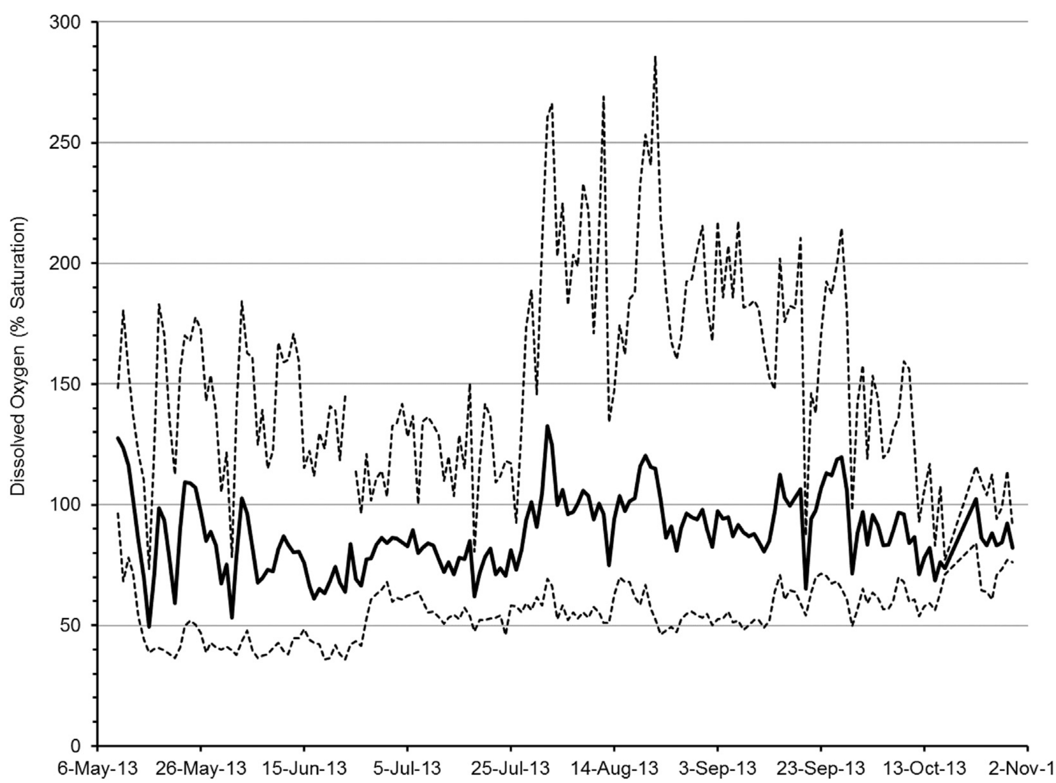

Figure 3. Mean (solid line) and maximum/minimum (dashed lines above/below mean) daily dissolved oxygen concentrations, as percent saturation, in 0.1-ha earthen ponds stocked with hybrid striped bass fed diets with 35, 38, or 41% intact digestible protein.

Table 6
Weekly water quality variable means (± SE) in ponds where hybrid striped bass (initial weight: 121.4 ± 0.8 g/fish; mean ± SE) were grown to market size on extruded diets containing one of three intact digestible protein (DP) levels (35, 38, 41%) and supplemented with the first three limiting amino acids (Met, Lys, Thr) at an ideal protein level of 45% hybrid striped bass muscle[a].

Response[b]	Intact DP (%)			
	35	38	41	*Pr* > *F*
NH_4-N	0.70 ± 0.06	0.65 ± 0.07	0.77 ± 0.06	0.523
NO_2-N	0.29 ± 0.05	0.23 ± 0.06	0.28 ± 0.05	0.673
NO_3-N	0.31 ± 0.08	0.26 ± 0.10	0.28 ± 0.08	0.964
PO_4-P	0.23 ± 0.03	0.19 ± 0.03	0.28 ± 0.03	0.176
Chl *a*	159.2 ± 25.0	205.6 ± 28.9	141.0 ± 25.0	0.257
NH_4-N spike	1.75 ± 0.35	2.00 ± 0.50	2.75 ± 0.35	0.086

[a] Values are least squares means of $N = 4$ replicate ponds of fish for diets 35% and 41% intact DP and 3 replicate ponds for the 38% intact DP diet.
[b] Total ammonia-nitrogen (mg NH_4-N/L); nitrite-nitrogen (mg NO_2-N/L); nitrate-nitrogen (mg NO_3-N/L); soluble reactive phosphorus (mg PO_4-P/L); chlorophyll *a* (mg/m^3); the number of times total ammonia-nitrogen (mg NH_4-N/L) exceeded 1.5 mg/L during "summer" (11 June to 14 September).

performance in our previous pond trial (Rawles et al., 2009) investigating the graded replacement of fish meal (FM) with pet-food grade poultry by-product meal (PBM) on an ideal protein (IP) basis. In that study, yields ranged from 5802 to 6697 kg/ha, average weights at harvest were 814-932 g, FCRs ranged from 1.99 to 2.15, PRE was 19–23%, and survival ranged from 95% to 99%. In contrast, Pine et al. (2008) also investigated the progressive replacement (0, 33, 67, 100%) of FM protein content with PBM on a crude protein basis in commercially extruded diets for pond-reared HSB; yields ranged from 4007 to 4592 kg/ha, average weights were 490–538 g, FCRs ranged from 2.31 to 2.55, PRE was 21–24%, and survival ranged from 81.6% to 86.3. Previously, D'Abramo et al. (2002) compared two-phase and three-phase pond production of HSB; yields ranged from 2837 to 3777 kg/ha, average weights were 553–631 g, FCRs ranged from 2.7 to 5.1, and survival ranged from 34.4% to 70.8%. In a follow-up study, D'Abramo et al. (2004) investigated the influence of stocking density and stocking weight on HSB performance in phase 2 (grow-out) of the two-phase pond production method; yields were 4506–5550 kg/ha, average weights were 529–634 g, FCRs ranged from 2.2 to 2.7, and survival ranged from 67.4–84.8%. Based on the strength of those results, many producers moved to the two-phase, instead of three-phase, production system. D'Abramo and Frinsko (2008) subsequently reported that typical commercial HSB yields ranged from 6500 to 7000 lbs./ac (≈7300 to 7900 kg/ha), FCR's ranged from 2.5 to 2.8 in larger ponds but were sometimes less in smaller (≤4 acres) ponds, and survival averaged 80%. Recent communication with producers indicates that current stocking densities have declined in portions of the industry, while average fish weight at stocking has increased. This has resulted in improved FCRs (< 2.5), increased survival to harvest (> 90%), and greater numbers of fish in larger market-size categories.

The lack of significant growth and feed performance differences, or whole body composition differences, compared to the results obtained in Rawles et al. (2012), suggest that digestible protein in HSB diets can be lowered significantly (100 g/kg) through multiple amino acid supplementation. Moreover, whole body protein retentions and amino acid retention efficiencies increased linearly by 3 percentage points (12% increase between 36 DP and 41 DP treatments) with decreasing intact DP. Similar results were obtained by Gaylord and Barrows (2009) in 20-g rainbow trout when diets were also supplemented with the first three limiting amino acids (Lys, Met, Thr) at the equivalent of 450 g/kg trout muscle. In a follow up study comparing diet performance based on different ingredient combinations ranging from animal products to different combinations of plant products, Gaylord et al. (2017) were also able to reduce total dietary protein from 450 g/kg to approximately 410 g/kg in 72-g rainbow trout supplementing Lys, Met, and Thr on an

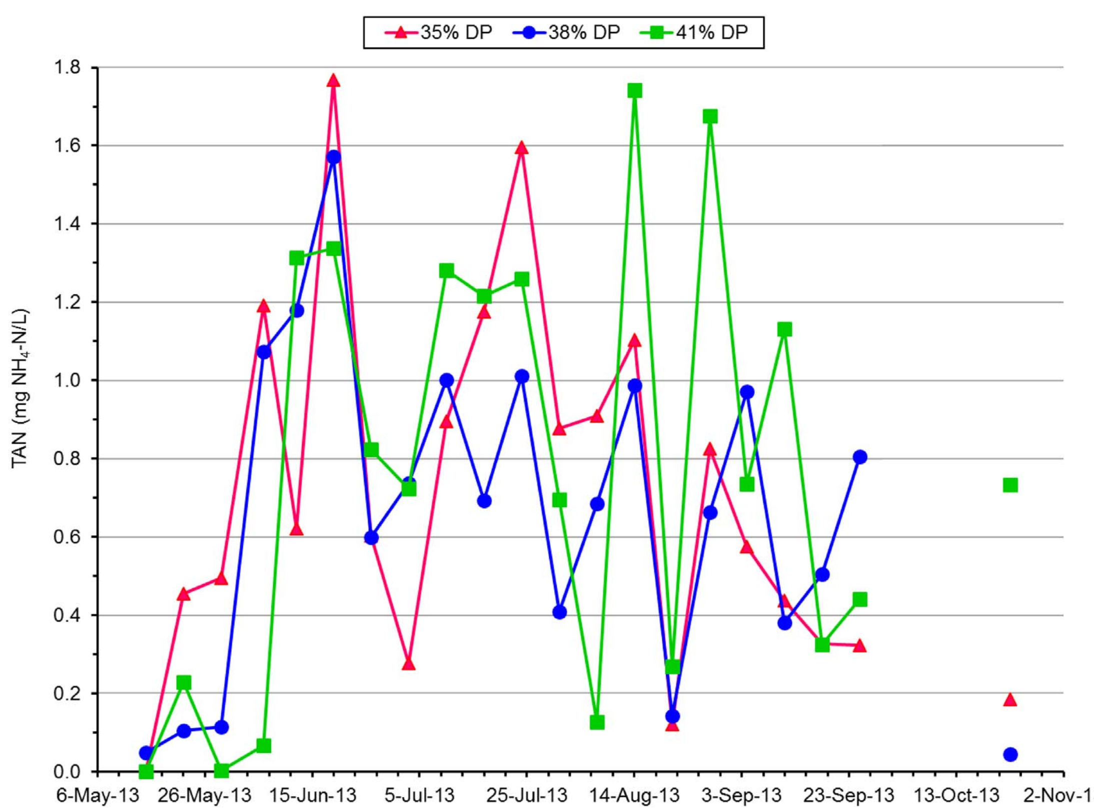

Figure 4. Mean weekly total ammonia-nitrogen concentrations in 0.1-ha earthen ponds stocked with hybrid striped bass fed diets with 35, 38, or 41% intact digestible protein (DP) ($N = 4$ replicate ponds for the 35% and 41% intact DP and $N = 3$ replicate ponds for the 38% intact DP diets). The gap in data corresponds to the 1–16 October 2013 shutdown of the USA government.

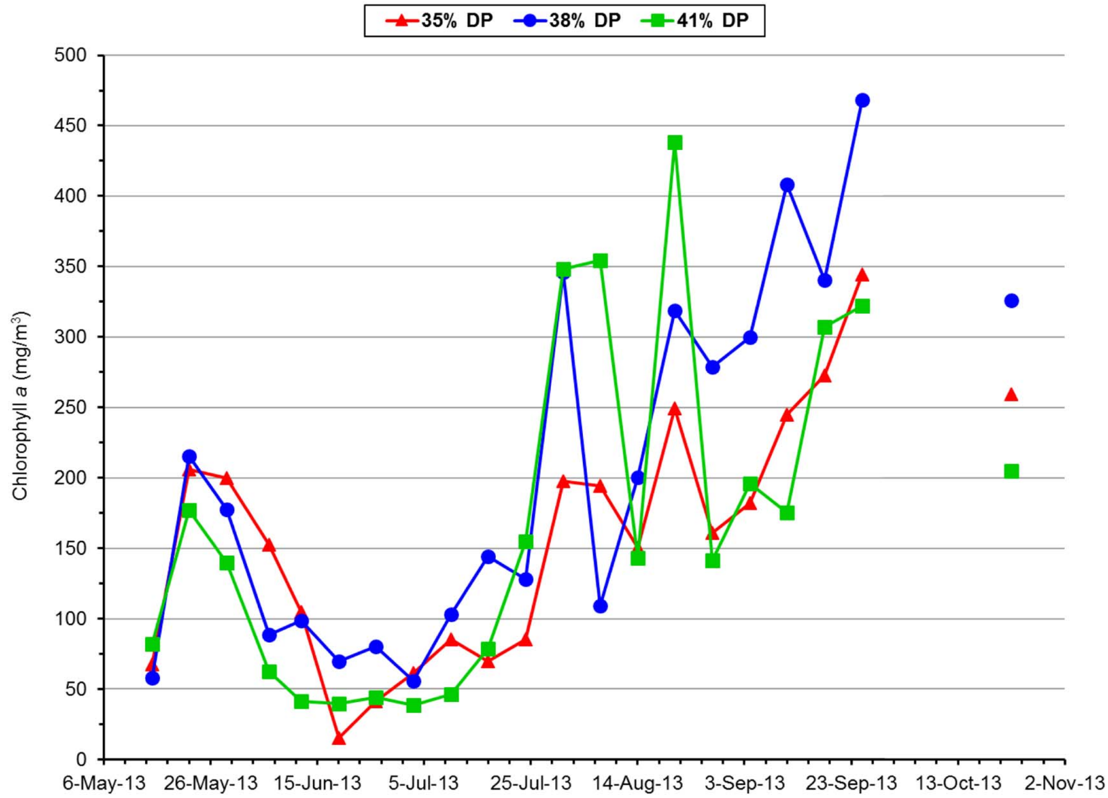

Figure 5. Mean weekly chlorophyll *a* concentrations in 0.1-ha earthen ponds stocked with hybrid striped bass fed diets with 35, 38, or 41% intact digestible protein (DP) (N = 4 replicate ponds for the 35% and 41% intact DP and N = 3 replicate ponds for the 38% intact DP diets). The gap in data corresponds to the 1–16 October 2013 shutdown of the USA government.

ideal basis.

The positive results we saw with the 35% DP diet in pond production are in sharp contrast to those of the 33% DP diet in our tank study (Rawles et al., 2012). The 33% DP diet consistently underperformed the higher DP (40 and 47%) diets in the tank study in terms of growth and lower ammonia excretion as a function of amount fed, or nitrogen fed per body weight. Moreover, the compositional data from the tank study also indicated insufficient diet energy to optimize growth and maintain body depots of energy at 33% DP and 18% dietary lipid at elevated temperature (30.5 °C), regardless of whether fish were fed at 80% or 100% of satiation. The difference in results is probably due to markedly different nutrient targets used for diet formulation in each of the two trials. The IP models for the tank study were the amino acid profiles of HSB muscle at each tested DP level, whereas the model for the current study was 45% HSB muscle regardless of the overall target DP of each test diet. In spite of the lack of compositional differences among fish fed the different pond diets, HSI increased linearly (P < 0.001) about a half a percentage point with decreasing DP, which is consistent with previous results that indicate HSI is a sensitive indicator of dietary differences in HSB at elevated temperature (Rawles et al., 2010, 2012). Since a decrease in DP corresponded to an increase in wheat flour in the current diets, the increase in HSI is probably related to some excess dietary carbohydrate being deposited in the liver as glycogen or fat. In contrast to our tank study, IPF was not statistically different (P = 0.213) or linearly related among treatments and suggests that dietary fat level was appropriate for the current formulas.

There is some indication that the 38% DP diet may have produced slightly better market size distributions in terms of more than expected medium (M) and very large (VL) fish and less than expected small (S) fish. In contrast, the 41% DP diet showed no advantage in terms of measured responses while producing fewer than expected very large (VL) fish. The difference in diet performance with respect to market-size distribution might be related to differences in amino acid profile. Koch et al. (2016) observed that the performance of Nile tilapia declined in a linear or quadratic fashion as one deviated from an ideal protein amino acid profile. As intact digestible protein decreased in the current study, the difference between the amino acid profile of the diet with respect to the ideal protein model (SS Dev – all A.A.; Table 2) also decreased. Hence, increased supplementation of the first three limiting amino acids with decreasing intact protein tended toward a more ideal balance. Interestingly, the 35% DP diet resulted in slightly more than expected fish in the very small (VS) market size category. This may be related to balance among unsupplemented amino acids since the amino acid profile in the 35% DP diet was also most distant (SS Dev – unsuppl. A.A.; Table 2) from that of the ideal protein model compared to the 38 and 41% DP diets. Unsupplemented amino acids include both those that are essential and nonessential and previous work has shown that the balance of both influence diet performance (Furuya et al., 2004; Gaylord and Barrows, 2009; Koch et al., 2016). Therefore, though differences between tabulated versus actual ingredient concentrations used for diet formulation may be small, they may be substantial enough in aggregate to influence fish performance to some extent. It is important, however, not to over emphasize market size distributions in relation to the test diet formulas fed in this study until an economic analysis determines whether the trade-off in increased feed costs of the 38% DP diet over the 35% DP diet is worth the potential gain in market price.

Diet lipid was lower (15%) than targeted (18%) in the test diets and raises the question of whether the lower lipid level affected protein retention efficiencies to some extent. When fish were fed to satiation in Rawles et al. (2012), significant interaction was found between lipid and DP with respect to PRE (Table 4); at 10% dietary lipid, protein retention increased from 25% to 30% at 47% DP and 33% DP, respectively. At 18% dietary lipid, PRE was 23% at 47% DP and significantly higher and statistically equal at 40% DP (32%) and 33% DP (29%). In other words, protein retention increased with decreasing DP, similar to the current results, but the effect was more pronounced in fish fed the lower lipid (10%) diets. Subsequent to our previous study, Jirsa et al. (2013) found that higher dietary lipid may be required for optimal heat stress tolerance given that heat-shock protein (HSP70 and HSP60) response in the liver and white muscle of white seabass, *Atractoscion nobilis*, increased with increasing dietary lipid up to a maximum of 18% lipid. Therefore, one might hypothesize that higher dietary fat might have potentially spared more protein for deposition in the current study, since this study included the hottest months of the growing season. Glencross et al. (2014) found a clear hierarchy in energy substrate preference in carnivorous barramundi fed diets of equal DE such that protein > lipid > starch; moreover, deposition efficiency of protein was highest (41%) when the contribution of digestible energy (DE) was biased toward lipid as opposed to protein (34%) or starch (33%). Subsequently, Jia et al. (2017) provided evidence in tissue isolates that amino acids are oxidized at a higher rate than carbohydrates and fatty acids in hybrid striped bass to provide ATP for liver, proximal intestine, kidney, and skeletal muscle. With the caveat that our previous results (Rawles et al., 2012) are for tank-reared fish up to 400 g while current results are for pond-reared, market-sized fish (> > 500 g), there is some indication that dietary fat at the targeted level might have potentially improved protein retention and reduced lipid deposition, since body fat (IPF) ranged from 6.4 to 7.5% in our previous study and 8.2 to 8.6% in the current study.

Food conversions in our pond study were markedly better (≈ 1.35) than pond studies currently in the literature and better than current commercial experience. This is a result of several efficiencies in our research setting that are not necessarily feasible in a commercial setting. First, our production cycle corresponded to the last 6-months only of a typical commercial two-phase, i.e., two-year, production cycle. Commercial growers start a year in advance of our scenario, stocking significantly smaller fingerlings, and grow them for two production seasons in order to reach market size. Therefore, commercial FCRs are based on a two-year process as opposed to our 6-month trial. Additionally, we stocked advanced juveniles (> 100 g) of exact known weights and numbers, grew them for 6 months to market size, and accounted for every fish at final harvest. Hence, the level of accuracy in the measurements of our inputs and outputs is probably much higher than commercial operations in general. Secondly, extreme care was paid to feeding research ponds that were significantly smaller than those in commercial operations. This allowed skilled, experienced personnel time to focus on insuring maximum feed intake and minimum wastage throughout the trial. Thirdly, in order to reduce feed loss due to wind or feeding activity driving pellets onto pond banks, research ponds were outfitted with large floating feed retainers that maximized fish opportunity to feed and minimized overfeeding.

Although fish were fed similar total quantities of feed and FCR's suggest minimal feed wastage, the total nitrogen delivered differed by dietary treatment. Since protein retention decreased linearly with increasing diet DP, we might have expected more correlations between pond TAN and dietary treatment. Nitrogen and phosphorus are excreted after consumed feed is metabolized, and although feed input was not correlated with TAN concentration, it was correlated positively with SRP concentration, which was positively correlated with TAN. Postprandial ammonia excretion by hybrid striped bass increases rapidly, peaks after about 6 h, and returns to its basal level after 24 h (Rawles et al., 2012). Although ammonia excretion by hybrid striped bass increases with respect to dietary protein content (Rawles et al., 2012), no effect on treatment TAN concentrations was observed in the present experiment, though there is some indication of DP influence on the number of TAN spikes over 1.5 mg/L (Table 6; $P = 0.086$). Nevertheless, the absence of stark treatment differences in water quality variables most likely resulted from the standardized nightly paddlewheel aeration in all ponds. Excretion of dietary soluble reactive phosphorus by hybrid striped bass averaged 11.6 mg PO_4-P/kg/d and was not affected by ration, or dietary DP or lipid content in a previous trial by Rawles et al., 2012. The absence of treatment differences for SRP was expected as all diets were formulated with equivalent digestible phosphorus levels. Concentrations of SRP remained low in treatment ponds throughout the study. In addition to phytoplankton uptake, the major sink for SRP ponds is adsorption by pond sediments (Masuda and Boyd, 1994).

Chlorophyll *a* concentration, which is an indicator of phytoplankton biomass, increased after stocking in response to fertilizer addition and concentrations corresponded to a moderate phytoplankton bloom (> 100 mg/m^3 chlorophyll *a*). Daily nutrient inputs from feed apparently were insufficient to sustain the phytoplankton bloom in all ponds beyond mid-June and the observed TAN spikes likely resulted because excretion of feed nitrogen by fish exceeded phytoplankton TAN uptake. Supporting evidence was the low chlorophyll *a* concentration during spike events and the negative correlation observed between TAN and Chl *a* concentrations. Algal uptake of TAN is the primary mechanism that controls TAN concentration in warm water aquaculture ponds (Hargreaves and Tucker, 1996; Hargreaves, 1998, 2006). Supplemental fertilizer addition through July and increasing feed nutrient inputs resulted in the increased chlorophyll *a* concentrations observed in all treatments. Increased chlorophyll *a* concentration in response to increased feed ration as the season progresses is common in intensively managed aquaculture ponds (Tucker et al., 1984; Boyd and Tucker, 1998).

Hybrid striped bass in a long-term study died when afternoon un-ionized ammonia concentration was 0.91 mg/L NH_3-N, but survival, growth, and feed conversion were unaffected by an afternoon concentration of 0.37 mg/L NH_3-N (Hargreaves and Kucuk, 2001). Calculated un-ionized ammonia concentrations during the TAN spike events ranged from 0.11 to 0.47 mg/L NH_3-N in the present study. These concentrations most likely will underestimate the maximum un-ionized ammonia concentration because afternoon pH was not measured. However, photosynthesis likely was low during most of the TAN spike events given the low chlorophyll *a* concentrations, which would moderate the increase in pH and un-ionized ammonia concentration. Periods of decreased chlorophyll *a* concentration are associated with increased TAN concentration, but decreased afternoon pH because photosynthetic activity is lower (Tucker et al., 1984). Thus, it is important to maintain good pond phytoplankton blooms.

The TAN spike events in the present study generally lasted no longer than 1 week, but in 5 ponds (2 each for the 35 DP and 41 DP diets) the spike events persisted for 2 to 4 weeks. Thus, it is possible that fish growth was affected as seen in the nominally lower final average weights for the 35 DP and 41 DP diets and the less favorable market-size distributions of those two treatments compared to the 38 DP diet. However, fish continued to feed throughout all TAN spikes and correlation analysis indicated no daily feed ration-TAN spike interaction. And, the similarity among diets of FCR indicates that consumed feed was used efficiently. Hargreaves and Kucuk (2001), citing Tucker et al. (1984), noted that elevated TAN concentrations in ponds rarely lasted longer than 5 to 10 d and the effect on fish growth would not be as great as the effect they observed in their study. However, Zhou and Boyd (2015) reported that for prolonged periods during the summer, TAN concentration remained high enough to be chronically toxic in some commercial catfish production ponds in west Alabama. Further research is needed on the effects on hybrid striped bass growth and health status of chronic exposure to high TAN concentration during pond culture.

In conclusion, while post-prandial TAN excretion by hybrid striped bass is known to increase with increasing dietary DP content (Rawles et al., 2012), pond ecosystem services (phytoplankton and microbial uptake, and soil adsorption), standardized nightly aeration, and setting a maximum daily feed ration in the current experiment did not allow stark treatment differences in water quality to manifest themselves. Results likely would be different if daily feed ration was to exceed quantities fed in the present experiment and nightly aeration was provided on an as needed basis, but this would need to be verified.

Finally, in addition to supplementing the first three limiting amino acids in the test commercial formulas, care was also taken to balance multiple ingredient/nutrient inputs in the test diets in order to reduce both confounding ingredient derived influences and potential deficiencies in other nutrients. Specifically, attention was given to available phosphorus, macro minerals and vitamins that are now considered important to overall diet balance (Barrows et al., 2008, 2010). Therefore, it appears that significant reduction can be made in digestible protein level in hybrid striped bass commercial diets using ideal protein diet formulation, a robust set of ingredient nutrient availabilities, and a higher level (45%) of muscle profile as the formulation target.

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